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Toxic, repellent and antifeedant effects of two aromatic plant extracts on the wheat granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae)

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Abstract
Wheat granary weevil, *Sitophilus granarius* L. is considered primary pest of stored grains. In the present study, methanol extract of dill *Anethum graveolens* L. and purple basil, *Ocimum basilicum* L. var. *purpurascens* were evaluated for their toxic, repellent and antifeedant effects against wheat granary weevil. Both the aromatic plants had repellent and antifeedant effects but dill leaf extract showed more toxic effect on the mortality (22.08%) for wheat granary weevil. Comparing the extracts, the higher repellency was observed in purple basil leaf extract on wheat granary weevil (46.55%). The results also showed that dill leaf extract had the higher antifeedant effects on wheat granary weevil having total coefficient of deterrency 154.20. The methanol leaf extract was more effective than those of water solvent. Insect mortality percent, coefficient of deterrency and in most of the cases, repellency rate increased proportionally with doses. Mortality percentage increased with the progress of time considering all the concentration.

Keywords: Botanical extract, toxicity, repellency, deterrency, wheat granary weevil, *Sitophilus granarius*

1. Introduction
Wheat plays a vital role in the diet of common people of developing countries. About 600 species of insects belonging to different families have been identified from stored products in various parts of the world. Among them the wheat granary weevil *Sitophilus granarius* L. is one of the most widespread and destructive primary insect pests of stored cereals such as wheat, maize, rice, oat, sorghum and barley. It causes loss to grain in storage either directly through consumption of the grain or indirectly by producing "hot spot" causing increase in moisture and thereby making grains more suitable for other stored grain pests [10, 5, 16].
Control of this insect population around the world is primarily dependent upon insecticides and fumigants which resulted in undesirable effects on non-target organisms, fostered, environment and human health concern. The increasing concern over the level of pesticide residues in food has encouraged researches to look for alternatives of synthetic pesticides [8].
Chemical control of insects in storage has been used with serious draw breaks [12]. The synthetic insecticides are expensive and have in many cases only produced moderate results along with major ecological damage [6].
In contrast, the low toxicity of botanical insecticides makes the processing and application of the product inexpensive. In many cases, the materials are locally available and affordable [2]. These have generated extraordinary interest in recent years as potential sources of natural insect control agents. There is an urgent need for safe, effective and bio-degradable pesticides with no toxic effects on non-target organisms.
The purpose of the study was to find out the effectiveness of some aromatic plants against wheat granary weevil for their insecticidal properties.

2. Material and Methods
2.1. Test insect
The wheat granary weevil, *Sitophilus granarius* L. was reared in a 1 L wide-mounted glass jars containing soft wheat grains. Mouth of the jars covered with a fine mesh cloth for ventilation and to prevent escape of the weevils. Cultures were maintained in an incubator at 27 ± 1 °C and 60 ± 5% relative humidity [3]. Insects used in all experiments were 1 to 7 day old adults.

All experimental procedures were carried out under the same environmental conditions as the cultures. The wheat granary weevil adults were obtained from the stock culture of the laboratory of the Plant Protection Department, Faculty of Agriculture, Ankara. The life cycle can be completed in as little 30 to 40 days during the culture conditions but takes considerable longer in cooler conditions. Adult granary weevils can live up to eight months and can produce up to four generations per year.

2.2. Test plants

In this study, bio-insecticide effects of aromatic leaf extracts from dill, *Anethum graveolens* L. and purple basil, *Ocimum basilicum* L. var. *purpurascens* were tested against wheat granary weevil, *S. granarius*. Dill and purple basil were obtained from a local market of Ankara.

2.3. Preparation of plant extracts

Fresh leaves of dill and purple basil were washed in running water and then they were dried in the oven at 60° C to prepare fine dust. Oven dried plant materials were prepared by pulverizing the dried leaves in a magnetic stirrer. A 25-mesh diameter sieve was used to obtain fine and uniform dust and preserved them into air tight glass jar, till their use in extract preparation. At first ten grams of each dust sample were taken in a 500 ml beaker and separately mixed with 150 ml of methanol and distilled water. Then the mixture was stirred for 30 minutes by a magnetic stirrer at 6000 rpm and left to stand for next 24 hours. The mixture was then filtered through a fine cloth and again through Whatman No. 1 filter paper. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent in a water bath at 80 °C and 60 °C temperature for water and methanol extracts respectively. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottles and stored in a refrigerator until their use for insect bioassays [13].

2.4. Contact toxicity test

The adult insects were chilled for a period of 12 minutes. The immobilized insects were picked up individually by using a small section tube. One µl solutions of different concentrations (5.0, 10.0, 15.0, and 20.0%) were applied to the dorsal surface of the thorax of each insect using a fine micro-pipette. After treatment, ten insects were transferred into 9 cm diameter Petri dish containing soft wheat grain. Test insects were examined daily and those that did not move or respond to gentle touch were considered as dead. Insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT). Observed mortalities or the insects were corrected by Abbott's formula [1].

Forty-five insects, in three replicates of 15 insects each, were treated at each dose. For control test, same number of insects was treated with solvent only. The mean values were separated by DMRT [4]. The corrected mortality data were analysed by probit analysis.

2.5. Repellency test

For repellency test, 9 cm diameter Petri dish was divided into three parts, treated, untreated and without grain part. Three grams of soft wheat grain placed in each chamber of Petri dish for wheat granary weevil. For application, 0.2 ml of solution of difference dose of each plant extract was applied to a side portion as uniformly as possible with a pipette. As a result, one side was treated and the other side was untreated test chamber. Ten insects were released at the central portion of each Petri dish and covered by a cover. There were three replications for each plant extract and dose. In the control side portion of Petri dish the grains were treated with solvent only. Then the number of insect on each chamber was counted at hourly intervals up to the sixth hour. The data were converted to express percent repulsion as positive values (repellency) and negative values (attractancy). The formula is:

PR (%) = (Nc-50) x 2 [14]

Where: PR. Percent repulsion, Nc. Percentage of weevils present in the control half.

Results of percent repulsion were analysed using ANOVA. The average values were then categorized, according to Mc Donald *et al.* [11].

Repellency rate (%)	**Class**
> 0.01 – 0.1	0
0.1 – 20	I
20.1 – 40	II
40.1 – 60	III
60.1 – 80	IV
80.1 – 100	V

2.6. Antife

The potential of the antifeedant effect of the aromatic plant extracts was determined by the feeding deterrency test described by Talukder and Howse [14]. For this purpose, wheat wafer discs were used as test food. The discs were saturated by dipping into either solvents only (control group) or into 10 mg/ml solution of each extract (treatment group) and were air-dried for 24 h. Wheat discs were then weighed and 12 weevils were placed on them. The discs were reweighed after 7 days. Food consumption of weevils was recorded under three conditions. On pure food (two untreated discs), on food with possibility of choice between one treated and one untreated disc choice test and on food with two treated discs non-choice test. Blank discs treated with the solvent but not offered to weevils were prepared. Discs were dipped for two different time periods, 15 and 30 seconds.

Disc weight loss, which was estimated as the amount of food consumed, was calculated by the formula of Serit *et al*., cited by Talukder and Howse [14].

FC = IWs - [(FWs IWb) / FWb]

Where

IW = Initial weight of disk after being treated with extract or solvent.

FW = Final weight of disc.

b = Blank disc treated with solvent and not offered to weevils.

s = Treated and control discs, on which weevils were released.

According to the amount of food consumed in the three different tests (control-control, treated-treated and control-treated) three feeding deterrent activity coefficients were calculated as absolute coefficient of deterrency, relative coefficient of deterrency and total coefficient of deterrency. The values of total coefficient of deterency serve as an index of antifeedant activity expressed on a scale between 0 – 200 for a compound with maximum activity. An index T of 150 – 200 was designated ++++; of 100 – 150 +++; of 50 – 100 ++, and of 0 – 50 +.

3. Results and Discussion

3.1. Contact toxicity and repellency effects

The results of the contact toxicity effects of dill and purple basil leaf extracts on wheat granary weevil *S. granarius* are showed in Table 1. Average mortality percentage of wheat granary weevil at 24, 48 and 72 hours after treatment (HAT) indicated that dill leaf extract (22.08%) possessed more toxic effect comparing purple basil leaf extract (10.65%). Mortality percentages were directly proportional to the time after treatment.

Table 1: Main mortality percentage of *S. granarius* treated with plant leaf extracts, solvents and different doses (HAT: Hours After Treatment).

Plant leaf extracts		Mortality (%)			
		24 HAT	**48 HAT**	**72 HAT**	**Average**
Dill		5.01e	23.23b	38.01a	22.08a
Purple basil		3.33e	9.32d	19.32c	10.65b
Plant leaf extracts	Solvents				
Dill	Water	5.00ef	15.66d	24.76b	15.14b
	Methanol	4.44ef	23.66b	43.66a	23.92a
Purple basil	Water	4.44f	9.00ef	16.00d	7.81c
	Methanol	3.33f	11.12e	20.01c	11.48bc
Plant leaf extracts	Doses (%)				
Dill	5.0	4.01	19.27	25.00	16.09
	10.0	9.42	26.17	40.22	25.27
	15.0	9.42	27.17	41.00	25.56
	20.0	9.40	27.00	43.43	26.61
Purple basil	5.0	2.21	3.03	11.11	5.45
	10.0	2.22	9.00	17.00	9.40
	15.0	5.01	9.99	22.94	12.64
	20.0	5.01	16.33	26.27	15.87

Data were evaluated by using Abbott's Formula.

The effects of different plant leaf extracts of different solvents on the mortality of insects are showed in Table 1. It was observed that the toxicity of different plant leaf extracts was influenced by the solvents. The highest toxic effect was observed in methanol extract of dill (43.66%) and the toxic effect was found in water extract of purple basil (16.00%) at 72 HAT. Average mortality percentage was also found directly proportional to the level of doses of plant leaf extracts (Table 1).

The repellency rates of insects were influenced by the concentrations of extracts shown in Table 2. Repellency rate did not increase proportionally with the doses. The highest mean repellency effect was found with 15% purple basil extract (52.24%).

Table 2: Repellent effect of different plant leaf extracts in different solvents and dose level on wheat granary weevil, *S. granarius*.

Plant leaf extracts		Repellency rate (%)					Mean repellency rate (%)	Repellency class
		1HAT	**2HAT**	**3HAT**	**4HAT**	**5HAT**		
Dill		32.71b	34.17b	50.26a	51.11a	55.50a	44.75b	III
Purple basil		27.53a	51.11a	55.37a	50.96a	47.80a	46.55a	III
Plant leaf extracts	Solvents							
Dill	Water	39.17f	32.61ef	39.47cf	45.91cf	49.76cd	41.38c	III
	Methanol	20.24g	30.37f	71.01ab	66.65ab	69.62ab	51.57b	III
Purple basil	Water	30.36f	35.61df	42.17c	45.72c	38.96ce	38.58c	II
	Methanol	18.58g	72.58a	63.54a	51.54b	52.94b	51.83a	III
Plant leaf extracts	Doses (%)							
Dill	5.0	29.87	18.76	48.12	39.15	44.13	36.00	II
	10.0	33.76	32.93	47.81	54.34	58.51	45.47	II
	15.0	23.71	30.38	46.96	57.80	46.52	41.07	III
	20.0	12.47	34.01	55.24	56.01	61.60	43.86	III
Purple basil	5.0	15.27	49.84	51.06	55.21	50.21	44.31	III
	10.0	30.17	60.56	58.14	53.47	57.51	51.97	III
	15.0	17.37	61.64	69.22	55.47	57.51	52.24	III
	20.0	19.34	47.98	49.76	58.47	61.61	47.43	III

From the interaction of plant leaf extract, dose and time, the mortality percentage at 72 HAT indicated that the dill 20% leaf extract possessed the highest toxic effect (43.43%) on wheat granary weevil. Same trend was also observed at 24 and 48 HAT. Average values indicated that mortality percentage differed significantly between plant leaf extracts, solvents and doses. The interaction effects of plant leaf extract-time were found to be significant at 1% level of probability. But the interaction effects of aromatic plant leaf extract-dose-time showed no significant effect on insect mortality.

The LC_{50} values of dill at 24, 48 and 72 HAT were 20.67%, 18.67% and 4.43% respectively and for purple basil were 70.67%, 28.56% and 19.76% respectively. Between two plant leaf extracts, LC_{50} values at 72 HAT indicated that the dill extract (4.43%) was highly toxic (Table 3).

Table 3. Relative toxicity of two plant leaf extracts against wheat granary weevil, *S. granarius.*

Plant leaf extracts	HAT	n: Insect	LC_{50} (%)
Dill	24	180	20.67
Purple basil		180	70.67
Dill	48	180	18.67
Purple basil		180	28.56
Dill	72	180	4.43
Purple basil		180	19.76

3.2. Antifeedant effects

The results of antifeedant effects of dill and purple basil plant leaf extracts on wheat granary weevil are presented Table 4. All the extracts had moderate inhibitory effects on the feeding activities of wheat granary weevil. Between the two aromatic plant leaf extracts tested, dill had the higher absolute deterrency (71.26) and purple basil had the lower effect (59.84). In case of relative deterrency, dill also showed the higher effect (77.16) followed by purple basil (62.22). Considering the total effects, the dill possessed the higher feeding deterrent effect (154.20), whereas purple basil possessed the deterrent effect (116.31). The differences of coefficient deterrent values among different plant extracts were found to be significant at 1% level of probability.

The results of present investigation on antifeedant effect influenced by solvents are presented in Table 4. Among the two solvents, the methanol extract of dill possessed the higher total feeding deterrent effect (138.45) and water extract of purple basil possessed lower total feeding deterrent effect (83.14).

Table 4. Antifeedant effect of different plant leaf extracts in solvent and different dose level on wheat granary weevil, *S. granarius.*

Plant leaf extracts		Co-efficient of deterrency (%)			
		Absolute	Relative	Total	Efficacy of extracts
Dill		71.26a	77.16a	148.42a	++++
Purple basil		59.84b	62.22b	122.06b	+++
Plant leaf extracts	**Solvents**				
Dill	Water	60.27c	65.66a	125.93c	+++
	Methanol	65.14b	69.74a	134.88a	+++
Purple basil	Water	41.41d	46.61d	88.02d	++
	Methanol	60.61b	65.76b	126.37b	+++
Plant leaf extracts	**Doses (%)**				
Dill	5.0	44.26e	49.20e	93.46e	++
	10.0	54.33d	59.26d	113.59d	+++
	15.0	66.43b	69.27b	135.70b	+++
	20.0	76.36a	79.14a	155.50a	++++
Purple basil	5.0	34.35f	38.10f	72.45f	++
	10.0	42.39e	46.10f	88.49e	++
	15.0	54.66d	59.16d	113.82d	+++
	20.0	60.16c	62.86c	123.02c	+++

Within column values followed by different letters are significantly different by DMRT

Mean antifeedant effect of different aromatic plant leaf extracts in different dose level on wheat granary weevil is shown in Table 4. The coefficient of deterrency increased proportionally with the increase of doses. The highest total coefficient of deterrency occurred in 20% concentration of dill leaf extract (162.16) and the lowest in 5% of purple basil leaf extract (77.70). The coefficient of deterrency values of different doses of plant leaf extracts was statistically significant at 5% level of probability.

It was observed that the dill leaf extract possessed the higher toxic effect on wheat granary weevil than purple basil and the LC_{50} value of dill extract for wheat granary weevil at 72 HAT indicated that dill was more toxic plant for *S. granarius*. Methanol extract of plant was more efficient than that of water extract. Insect mortality percentage, coefficient of deterrency and in most the cases, repellency rate increased proportionally with doses. Mortality percentage increased but the repellency effect decreased with the progress of time. The use of aromatic botanical materials as insecticides will benefit agricultural sector. They are not only of low cost, but have no environmental impact in term of insecticidal hazard.

Saljoqi *et al*. [13] tested the repellent and lethal potential of ethanol extracts of six plants (*Melia azdaracha*, *Myrtus communis, Mentha longifolia, Pegnum harmala* and *Cymbopogon citrates*) against rice weevil, *Sitophilus oryzae*. They reported that extracts of bakain leaves recorded maximum repellency index with non-significance differences with hobulus and mint as compared with the untreated control. These were followed by bakain leaves and lemon grass. Harmal recorded the least effect of repellency against *S. oryzae*. Viglianco *et al*. [15] carried out investigation on some plant extracts to evaluate their repellency and feeding deterrency to control *S. oryzae*. They used three plants (*Alaysia polystacthia, Solanum argentium* and *Tillandsia recurvata*) for their studies. They reported hexane extract of *S. argentium* with strongest repellent effects (class IV) against *S. oryzae* whereas, ethanol and chloroform extracts of all plants recorded moderate repellency. Other scientists reported significan repellency some aromatic plant leaf extracts while working with different insects pests of stored cereals [7, 9, 15]. Although I recorded significant repellency of dill extract however, it was found least repellent among all tested plants against *S. granarius* that might be due to its relative resistance.

4. Conclusion

Efficacy of different plant extracts was evaluated for their growth inhibiting and grain protecting action against wheat granary weevil, *S. granarius*. The reduction of first adult emergence of granary weevil by using the extract of leaf of dill and purple basil can control the weevil easily. The plant extract will be very economic and non-toxic for human and environment will remain safer. Considering cost effectiveness, easy preparation, easy usage technology and environment-friendly advantages plant extracts can be the most important component of integrated pest management in controlling granary weevil of storage. On the other hand, wheat is an important agricultural crop, especially, for solving the world hunger problem.

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